BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic representation of the DNA methyltransferase gene (above) and the positions of the various 20-mer phosphorothioate antisense oligonucleotides (shown as filled circles) on the gene, with the positions of two non-limiting synthetic oligonucleotides, MG88 (SEQ ID NO: 1) and MG98, SEQ ID NO: 2 highlighted.

Figure 2 is a representation of autoradiographs of Western blotting analyses showing that treatment with 40 nM or 80 nM of a representative, non-limiting antisense oligonucleotide, MG88 (SEQ ID NO: 1), results in an inhibition of MeTase protein expression in both A549 and T24 cells.

Figure 3A is a representation of autoradiographs of a series of immunoprecipitations followed by Western blotting analyses of T24 cell lysates showing that $p16^{ink4}$ protein levels (upper panel) increase as DNA MeTase protein levels (lower panel) decrease following treatment of the cells for 3, 5, 8, or 10 days with 40 or 75 nM of a representative, non-limiting antisense oligonucleotide of the invention, MG88 (SEQ ID NO: 1), where HeLa cells served as a positive control for $p16^{ink4}$ protein expression.

Figure 3B is a graphic representation of $p16^{ink4}$ protein levels normalized to cell number of T24 cells treated with 40 nM or 75 nM MG88 (SEQ ID NO: 1) for 3, 5, 8, and 10 days.

Figure 4 is a representation of an autoradiograph of a Western blotting analysis of T24 cells treated with 40 nM or 75 nM of a representative non-limiting antisense oligonucleotide of the invention, MG88 (SEQ ID NO: 1), using an antibody recognizing all phosphorylated forms of Rb.

Figure 5 is a representation of autoradiographs of a series of Western blotting analyses of T24 cell lysates prepared 3, 5, or 7 days after cessation of a 10 day treatment of the cells with lipofectin only, 40 nM of MG88 (SEQ ID NO: 1), or 40 nM or 75 nM of control oligonucleotide MG208 (SEQ ID NO: 4), demonstrating that between 5-7 days post-treatment, DNA MeTase protein expression is restored and $p16^{ink4}$ protein expression is diminished.

Figure 6 is a graphic representation of the quantitation of DNA MeTase and $p16^{ink4}$ protein levels in T24 cells during ten days of treatment with MG88 (SEQ ID NO: 1) and 7 days (*i.e.*, days 11-17) post-treatment periods, demonstrating the inverse

relationship between p16 protein levels and DNA MeTase protein levels in these cells.

Figure 7 is a representation of a series photographs of showing the methylation-specific PCR (MSP) products resolved on 2% agarose gels of the (SeQ ID No: 1) (SeQ ID No: 4) p16^{ink4} gene promoter from T24 cells treated with 40 or 75 nM of MG88 or MG208 of 3, 5, 8, or 10 days using PCR primers specific for methylated p16^{ink4} (M lanes) or unmethylated p16^{ink4} (U lanes), demonstrating that demethylation of p16^{ink4} occurred only in MG88 treated cells after at least 3 days of treatment.

Figure 8 is a diagrammatic representation of the p16^{ink4} proximal promoter from T24 cells showing the methylation patterns after 0, 3, or 5 days of treatment of the cells with MG88 (SeQ ID No: 1) (left) or control oligonucleotide MG208 (SeQ ID No: 4) (right), demonstrating that reduced methylation occurred only with MG88 treatment. Day 3 post-treatment methylation patterns are shown at the bottom of the figure.

Figure 9A is a representation of a photograph of the results of bisulfite sequencing of the p16^{ink4} gene promoter in p16^{ink4}-expressing clone MG88 (SeQ ID No: 1) C4-5 30 days following cessation of a 5 day treatment with 75 nM MG88, demonstrating that all CpG sites evaluated were not methylated in this clone even after 30 days in culture post-MG88 treatment.

Figure 9B is a graphic representation showing a growth curve of T24 cells during treatment (days 0-5) and post-treatment (days 6-18) with lipofectin only (squares), 75 nM MG88 (SeQ ID No: 1), a non-limiting representative antisense oligonucleotide of the invention (circles), or 75 nM or control oligonucleotide, MG208 (SeQ ID No: 4) (triangles), demonstrating the anti-proliferative effect of MG88.

Figure 9C is a graphic representation showing a growth curve during post-treatment of T24 cell clones following a five day treatment with MG88 (SeQ ID No: 1) (clone 4-5, triangles), MG208 (SeQ ID No: 4) (clone 2-4, squares), or lipofectin only (clone 5, diamonds). The inserted representation of two autoradiographs show the p16^{ink4} protein level in each of the clones of days 36 and 49 post-treatment.

Figure 10A is a representation of autoradiographs of Western blotting analyses of p21^{WAF1}, MeTase, and α-actin protein levels in T24 cells treated for 24 hours (left panels) or 48 hours (right panels) with lipofectin only, or 40 nM or 75

nM of MG88 or MG208, demonstrating that $p21^{WAF1}$ protein expression is induced by inhibition of MeTase expression.

Figure 10B is a representation of autoradiographs of Western blotting analyses showing the dose-response of $p21^{WAF1}$ protein levels in T24 cells treated for 24 hours with 20 nM, 40 nM, or 80 nM of MG88 or MG208. Actin protein levels are shown as a control for protein loading.

Figure 11 is a representative of an autoradiograph showing a Northern blotting analysis of RNA from T24 cells treated for 24 or 48 hours with MG88 or MG208.

Figure 12 is a representation of an autoradiograph of a Western blotting analysis showing the reactivation of p16 expression in T24 cells following treatment for three days with increasing concentrations of a representative, non-limiting DNA MeTase protein effector, 5-aza-dC.

Figure 13 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic antisense oligonucleotide (MG88) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention; the three panels show varying combinations and concentrations of representative antisense oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 14 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic antisense oligonucleotide (MG98) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention; the three panels show varying combinations and concentrations of representative oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 15 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic oligonucleotide (MG88) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention at low concentrations; the three

panels show varying combinations and concentrations of representative oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 16 is a graphic representation showing the ability of a representative, nonlimiting, synthetic antisense oligonucleotide (MG98 (SeQ ID No:2)) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit T24 human bladder cancer cell growth in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the antisense oligonucleotides or only the DNA MeTase protein effectors.

Figure 17 is a graphic representation showing the synergistic inhibition of T24 human bladder cancer cell growth after treatment for seven days with lipofectin only (first bar from the left); 1 μM of a representative, nonlimiting, DNA MeTase protein effector, 5-aza-dC (second bar from the left); 40 nM of control synthetic oligonucleotide MG207 (SeQ ID No:3) (third bar from the left); 40 nM of a representative nonlimiting synthetic MeTase antisense oligonucleotide, MG98 (SeQ ID No:2) (fourth bar from the left); MG207 plus 5-aza-dC (fifth bar from the left); or MG98 plus 5-aza-dC (sixth bar from the left).

Figure 18 is a graphic representation showing the ability of a representative, nonlimiting, synthetic oligonucleotide (MG98 (SeQ ID No:2)) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit A549 human non-small cell lung cancer cell growth in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the oligonucleotides or only the DNA MeTase protein effectors.

Figure 19 is a graphic representation showing the ability of a representative, nonlimiting, synthetic oligonucleotide (MG98 (SeQ ID No:2)) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit Colo 205 human colon cancer cell growth (expressed as tumor volume over time) in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the oligonucleotides or only the DNA MeTase protein effectors.

Figure 20A is a graphic representation showing the inhibition of Colo 205 tumor cell growth in nude mice following treatment of the mice with saline (diamond); 0.5 mg/kg of a representative, nonlimiting MeTase synthetic

(SeQ ID No: 2)
oligonucleotide, MG98 (square); 0.1 mg/kg of a representative, nonlimiting, DNA MeTase protein effector, 5-aza-dC (triangle); or a combination of both MG98 plus 5-aza-dC (X).

Figure 20B is a graphic representation showing the inhibition of Colo 205 human colon cancer cell growth (expressed as final tumor volume) by a representative, nonlimiting, synthetic oligonucleotide (MG98) (SeQ ID No: 2) and by a representative, nonlimiting DNA MeTase protein effector (5-aza-dC) according to the invention, in a synergistic fashion, resulting in an statistically increased inhibitory effect ($p<0.05$) as compared to that observed using either only oligonucleotide, protein effector, or saline.

Figure 21 is a schematic diagram showing a series of FACS histogram analyses of T24 cells treated with a representative, nonlimiting, synthetic oligonucleotide (MG88) (SeQ ID No: 1) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention, at different schedules. The upper panel of histograms shows cells treated on schedule A, where MG88 is administered before 5-aza-dC. The lower panel of histograms shows cells treated on schedule B, where 5-aza-dC is administered before MG88.

Figure 22 is a representation of an autoradiograph of the Western blotting analysis showing the synergistic inhibition of thymidylate synthase protein expression in T24 cells using the combination of a representative, nonlimiting, synthetic antisense oligonucleotide (MG2605) (SeQ ID No: 71) and a representative, nonlimiting TS protein effector (5-FU) according to the invention.

Figure 23 is a schematic diagram showing a series of FACS histogram analyses of T24 cells treated with a representative, nonlimiting synthetic thymidylate synthase antisense oligonucleotide and/or a representative non-limiting TS protein effector (5-FU) according to the invention. The top histograms shows cells treated with lipofectin only; the second histogram shows cells treated with 25 nM mismatch control oligonucleotide; the third histogram from the top shows cells treated with 25 nM of the TS antisense oligonucleotide, MG2605; (SeQ ID No: 71) the fourth histogram from the top shows cells treated with 500 nM of 5-FU; the fifth histogram from the top shows cells treated with 5-FU plus mismatch

oligonucleotide; and the sixth histogram (*i.e.*, the bottom histogram) shows cells treated with 5-FU plus the TS antisense oligonucleotide, MG2605.

Figure 24A is a graphic representation showing the percentages of T24 cells in the G_1 phase (gray bars; section M2 on inserted histograms) S phase (black bars; section M3 on inserted histograms), and G_2/M phase (white bars; section M4 on inserted histogram) following treatment with 25 nM of a representative, nonlimiting TS antisense oligonucleotide MG2605 (SEQ ID NO: 71), 25 nM of control oligonucleotide MG2606 (SEQ ID NO: 81), 5 μM of a representative nonlimiting TS protein effector, 5-FU, or a combination of oligonucleotide plus 5-FU.

Figure 24B is a graphic representation of the number of T24 cells remaining following treatment with following no treatment; or treatment with 25 nM of a representative, nonlimiting TS antisense oligonucleotide MG2605 (SEQ ID NO: 71), 25 nM of control oligonucleotide MG2606 (SEQ ID NO: 81), 5 μM of a representative nonlimiting TS protein effector, 5-FU; or a combination of MG2605 or MG2606 plus 5-FU.

Figure 25 is a representation of an autoradiograph of the Western blotting analysis showing the synergistic induction of $p21^{WAF1}$ by the combination of a representative, nonlimiting, synthetic HDAC antisense oligonucleotide and a representative, nonlimiting HDAC protein effector (TSA) according to the invention.